BIODEGRADATION OF EPICHLOROHYDRIN

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Inventor(s): ASANO YASUHISA + (ASANO YASUHISA)
Applicant(s): NISSAN CHEMICAL IND LTD + (NISSAN CHEM IND LTD)
Classification:
- international: ***C02F1/58; C02F3/34; C12N1/20;*** **C02F1/58; C02F3/34; C12N1/20;** (IPC1-7): C02F1/58; C02F3/34; C12N1/20
- European:
Application number: JP19990211863 19990727
Priority number(s): JP19990211863 19990727

Abstract of **JP 2001037469 (A)**

PROBLEM TO BE SOLVED: To provide a new kind of potent microorganisms intended for biodegrading epichlorohydrin, and a method for biodegrading epichlorohydrin using the above microorganisms, in particular for degrading epichlorohydrin contained in effluents and waste liquors. SOLUTION: This method for biodegrading epichlorohydrin comprises using a strain selected from each new kind of microorganisms Arthrobacter ureafaciens 3CL7 (FERM P-17450) strain, Microbacterium sp. CL13 (FERM P-17452) and Erwinia carotovora 4CL5 (FERM P-17451) strain.

L1 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2011 ACS on STN
AN 2001:109916 HCAPLUS Full-text
DN 134:136159
TI Epichlorohydrin-degrading microorganism
IN Asano, Yasuhisa
PA Nissan Chemical Industries, Ltd., Japan
SO Jpn. Kokai Tokkyo Koho, 6 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2001037469	A	20010213	JP 1999-211863	19990727 <-
PRAI JP 1999-211863		19990727		

AB Epichlorohydrin in wastewater and liquid is degraded efficiently with microorganisms selected from Arthrobacter ureafaciens, Microbacterium, and Erwinia carotovora. The physiol. and morphol. characteristics of these microorganisms were also given.

L2 ANSWER 1 OF 3 WPIX COPYRIGHT 2011 THOMSON REUTERS on STN
AN 2001-337957 [200136] WPIX Full-text
DNC C2001-104764 [200136]
TI Decomposition of waste water containing epichlorohydrin, involves treating
with novel strains of Arthrobacter ureafaceiens, Microbacterium species
and Erwinia carotovora
DC D15; D16; E13
IN ASANO Y
PA (NISC-C) NISSAN CHEM IND LTD
CYC 1
PI JP 2001037469 A 20010213 (200136)* JA 6[0]
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ADT JP 2001037469 A JP 1999-211863 19990727
PRAI JP 1999-211863 19990727
IPCR C02F0001-58 [I,A]; C02F0001-58 [I,C]; C02F0003-34 [I,A]; C02F0003-34
[I,C]; C12N0001-20 [I,A]; C12N0001-20 [I,C]
FCL C02F0001-58 A; C12N0001-20 A; C12N0001-20 D; C12N0001-20 F; C02F0003-34 Z
(ZAB)
FTRM 4B065; 4D038; 4D040; 4D038/AA08; 4B065/AA13.X; 4B065/AA25.X; 4B065/AA32.X;
4D038/AB09; 4D038/AB14; 4B065/AC12; 4B065/AC20; 4B065/BB01; 4B065/BB03;
4D038/BB13; 4D038/BB19; 4B065/BB29; 4B065/BC02; 4B065/BC03; 4B065/BC26;
4B065/BD15; 4B065/BD50; 4B065/CA56; 4D040/DD03; 4D040/DD12
AB JP 2001037469 A UPAB: 20050525
NOVELTY - Waste water containing epichlorohydrin is decomposed using the novel microorganism Arthrobacter ureafaceiens 3CL7 (FERM P-17450) strain, Microbacterium sp. CL13 (FERM P-17452) strain and Erwinia carotovora 4CL5 (FERM P-17451) strain, is new.
USE - For treating waste water and liquid waste containing epichlorohydrin ejected from chemical plants.
ADVANTAGE - The method enables effective decomposition of epichlorohydrin in the waste liquid using novel strains of microorganism.
MC CPI: D04 A01J; D04 A01P; D04 A05; D04 B; D04-B06; D05-A04A; D05-H04;
D05 H08; D05 H13; E07 A03A; E11 Q02

PATENT ABSTRACTS OF JAPAN

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C02F 3/34

(21)Application number : **11-211863**
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(71)Applicant : **NISSAN CHEM IND LTD**
(72)Inventor : **ASANO YASUHISA**

(54) BIODEGRADATION OF EPICHLOROHYDRIN

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a new kind of potent microorganisms intended for biodegrading epichlorohydrin, and a method for biodegrading epichlorohydrin using the above microorganisms, in particular for degrading epichlorohydrin contained in effluents and waste liquors.
SOLUTION: This method for biodegrading epichlorohydrin comprises using a strain selected from each new kind of microorganisms Arthrobacter ureafaciens 3CL7 (FERM P-17450) strain, Microbacterium sp. CL13 (FERM P-17452) and Erwinia carotovora 4CL5 (FERM P-17451) strain.

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(51)Int.Cl.⁷		識別記号	FI			テーマコード（参考）
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					Ｄ	４Ｄ０４０
Ｃ０２Ｆ	1/58		Ｃ０２Ｆ	1/58	Ａ	
	3/34	ＺＡＢ		3/34	ＺＡＢＺ	

審査請求　未請求　請求項の数４　ＯＬ　（全６頁）

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(22)出願日　平成11年７月27日(1999.7.27)

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富山県射水郡小杉町黒河5180

Ｆターム(参考)　4B065 AA13X AA25X AA32X AC12
AC20 BB01 BB03 BB29 BC02
BC03 BC26 BD15 BD50 CA56
4D038 AA08 AB09 AB14 BB13 BB19
4D040 DD03 DD12

(54)【発明の名称】　エピクロロヒドリンの微生物分解

(57)【要約】
【課題】　エピクロロヒドリンの分解のための強力な新規微生物及びその微生物を利用するエピクロロヒドリンの分解、特に排水、廃液中に含有されるエピクロロヒドリンを分解する方法の提供
【解決手段】　新規な微生物アルスロバクター・ウレアファシエンス３Ｃ１.７（Arthrobacter ureafaciens 3C1.7）ＦＥＲＭ　Ｐ　１７４５０菌株、ミクロバクテリウム・スピーシズＣ１.１３（Microbacterium sp. C1.13）ＦＥＲＭ　Ｐ　１７４５２菌株又はエルヴィニア・カロトボラ４Ｃ１.５（Erwinia carotovora 4C1.5）ＦＥＲＭ　Ｐ－１７４５１菌株から選ばれる菌株を用いたエピクロロヒドリンの分解方法。

【特許請求の範囲】
【請求項１】　アルスロバクター・ウレアファシエンス３ＣＬ７（Arthrobacter ureafaciens 3CL7）ＦＥＲＭ　Ｐ　１７４５０菌株、ミクロバクテリウム・スピーシズＣＬ１３（Microbacterium sp. CL13）ＦＥＲＭ　Ｐ－１７４５２菌株又はエルヴィニア・カロトボラ４ＣＬ５（Erwinia carotovora 4CL5）ＦＥＲＭ　Ｐ　１７４５１菌株から選ばれる菌株を用いたエピクロロヒドリンの分解方法
【請求項２】　アルスロバクター・ウレアファシエンス３ＣＬ７（Arthrobacter ureafaciens 3CL7）ＦＥＲＭ　Ｐ－１７４５０菌株
【請求項３】　ミクロバクテリウム・スピーシズＣＬ１３（Microbacteriumsp. CL13）ＦＥＲＭ　Ｐ　１７４５２菌株。
【請求項４】　エルヴィニア・カロトボラ４ＣＬ５（Erwinia carotovora 4CL5）ＦＥＲＭ　Ｐ　１７４５１菌株。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明はエピクロロヒドリンの製造又はエピクロロヒドリンを使用する工程から排出されるエピクロロヒドリンを含む排水の処理する方法に関する。エピクロロヒドリンは、化学品の合成原料として大量に製造・消費されている
【０００２】
【従来の技術】エピクロロヒドリンの様なハロゲン炭素の結合を持つ有機化合物の工業的な規模での処理には特別な難しさがある。すなわち、炭素－ハロゲン共有結合が安定であるために、これを切断するのに多大のコストを要する事である。従来これらハロゲン化された有機物質は化学的・物理的方法及び生物的方法により分解される。ここで使用される物理的方法としてはたとえば活性炭による吸着、及び抽出法である。しかし、この方法はハロゲン化有機化合物で汚染された大量の活性炭や抽出物が生じるという問題があり、これらの処理に多大の費用がかかる。次に化学的な処理としては多くの場合酸化的雰囲気で高温、高圧条件でハロゲン化合物を分解する方法である。例えば、特開平６－３２０１９４号公報及び米国特許５４７８４７２号公報に示されている様に、排水を熱的アルカリ処理後、セルロモナス属細菌等のグラム陽性菌及びアルカリゲネス属細菌等のグラム陰性菌を使用して生物処理を行っている。さらに、場合により化学的酸化処理を行っている。しかしこの方法では特別な装置が必要なエネルギーコストが大きく、経済的な処理法とは言えない。特開昭５０－０３２７６７号公報には電気分解したのちにイオン交換膜で処理する方法も示されている。がこれも同様に多くのエネルギーを必要とする。その他、有機ハロゲン化合物と高い反応性を持つ金属又は金属水素化物で処理する方法もあるが、これもコストが高く又分解率も十分とは言えない。
【０００３】以上の様に現状では、経済性のあるすぐれたエピクロロヒドリンの分解法がないために多大のエネルギーを使用して廃液の焼却処理を余儀なくされている。しかし、近年のようにダイオキシンの発生が世界的に大きな問題になっており、燃焼する事も難しくなっており、環境に負荷の少ない、経済的な処理法が求められていた
【０００４】マリケンら（Eur. J. Biochem. 202, 1217（1991））は、アグロバクテリウム・ラジオバクター（Agrobacterium radiobacter AD1）が、エピクロロヒドリンを単一の炭素源として生育することについて報告しているが、本発明の微生物とは異なる。またエピクロロヒドリンの培養濃度は、5mMまでである。笠井ら（Agric. Biol. Chem. 54, 3185 (1990)）は、シュウドモナス・スピーシズ（Pseudomonas sp. OS-K-29）が、エピクロロヒドリンを単一の炭素源として生育することを述べているが、本発明の微生物とは異なる。またエピクロロヒドリンの培養濃度は、約25mM（0.2%）までである。
【０００５】
【発明が解決しようとする課題】本発明の目的は、エピクロロヒドリンの分解のための強力な新規微生物及びその微生物を利用するエピクロロヒドリンの分解、特に排水、廃液中に含有されるエピクロロヒドリンを分解する方法を提供することである。
【０００６】
【課題を解決するための手段】本発明者は、エピクロロヒドリンの新しい生物化学的処理方法を開発するために、自然界に菌株を広範囲にスクリーニングしたところ、いくつかの細菌がエピクロロヒドリンを分解することを見出した。すなわち、日本の山口県の土壌中から、高濃度のエピクロロヒドリンを分解する新たな菌種を取得し、これら菌株をエピクロロヒドリンを含む水性媒体と接触させることによるエピクロロヒドリンの生物化学的処理方法を提供することにより解決される。
【０００７】
【発明の実施の形態】以下、本発明を詳細に説明する。
（１）微生物
本発明において新たに分離された３ＣＬ７株、ＣＬ１３株及び４ＣＬ５株は、山口県下の土壌からスクリーニングして単離したものであり、高いエピクロロヒドリン分解活性を有する。これら新菌株は、以下に示すものである
【０００８】アルスロバクター・ウレアファシエンス３ＣＬ７株は、工業技術院生命工学工業技術研究所にＦＥＲＭ　Ｐ　１７４５０として寄託されている。
【０００９】　ミクロバクテリウム・スピーシズ　ＣＬ１３は、工業技術院生命工学工業技術研究所にＦＥＲＭ　Ｐ　１７４５２として寄託されている。
【００１０】エルヴィニア・カロトボラ４ＣＬ５は、工

業技術院生命工学工業技術研究所にＦＥＲＭ　Ｐ－１７　４５１として寄託されている。以下に、これら新菌株の菌学的性質を示す。

【００１１】

	３ＣＬ７株	ＣＬ１３株	４ＣＬ５株
（ａ）形態的性質			
１　細胞の形	桿菌	桿菌	桿菌
２　細胞の大きさ（μｍ）	０．６×１	０．６×２	０．８×１
２　多形成の有無	－	＋	－
３　運動性の有無			＋
４　胞子の有無			－
（ｂ）培養的性質			
１　肉汁寒天平板培養（３０℃、３日間）			
イ）コロニー形状（直径、ｍｍ）	２	２	３
ロ）コロニーの形	円形	円形	円形
ハ）コロニーの表面の形状	平滑	平滑	平滑
ニ）コロニーの隆起状態	低凸状	低凸状	低凸状
ホ）コロニーの周縁	全縁なめらか	全縁なめらか	全縁なめらか
ヘ）コロニーの色調	黄色	黄色	クリーム
ト）コロニーの透明度	不透明	不透明	不透明
チ）コロニーの光沢	あり	あり	鈍光沢
リ）可溶性色素の生成	なし	なし	なし
２　肉汁寒天斜面培養（３０℃、３日間）			
イ）生育の良否	良好	良好	良好
ロ）コロニーの光沢	あり	あり	あり
３　肉汁液体培養（３０℃、７日間）			
イ）表面の生育	あり	あり	あり
ロ）濁度	濁る	濁る	濁る
ハ）沈殿	粉状	粉状	粉状
ニ）ガス発生	なし	なし	なし
４　肉汁ゼラチン（３０℃、７日間）			
ゼラチン液化	＋		－
５　リトマス・ミルク			
（３０℃、７日間）	青変	赤変	青変
（ｃ）生理学的性質			
１　グラム染色	＋	＋	－
２　硝酸塩の還元			＋
３　脱窒反応			－
４　ＭＲテスト			－
５　ＶＰテスト			＋
６　インドール生成			＋
７　硫化水素の生成			－
８　デンプンの加水分解	－		－
９　クエン酸利用			
イ）koser	＋	＋	＋
ロ）Christensen	＋	＋	＋
10　色素の生成			
イ）king　A培地			－
ロ）king　B培地			－
11　ウレアーゼ			－
12　オキシダーゼ			－
13　カタラーゼ	＋	＋	＋

14　生育の範囲			
(イ) pH	5　9	6　9	6－8
(ロ) 温度			
30℃	+	+	+
37℃	+		−
41℃	+		−
15　酸素に対する態度	通性嫌気性	好気性	通性嫌気性
16　O－Fテスト(グルコース)	酸化的	酸化的	発酵的
17　糖類からの酸及びガスの生成			
	酸　ガス	酸　ガス	酸　ガス
1　L－アラビノース			+ −
2　D－キシロース			+ −
3　D－グルコース			+ +
4　D－マンノース			+ −
5　D－フラクトース			+ −
6　D－ガラクトース			+ −
7　マルトース			+ −
8　シュークロース	−	+	+ −
9　ラクトース			+ −
10　トレハロース			+ −
11　D－ソルビット			+ −
12　D－マンニット			+ −
13　グリセリン	−		+ −
14　デンプン	−	−	+ −
15　ラフィノース			+ −
16　イヌリン	−	−	+ −
17　D－リボース		−	+ −
18　ソルボース		+	+ −
19　カルボキシメチルセルロース			− −
20　グリコーゲン			− −
(ロ) その他の諸性質			
ビタミン要求性	なし	なし	なし
アルギニンの分解	+	+	−
ヒスチジノールの分解			−
ニコチンの分解			−
耐塩性　5%	+		+
7%	+		−
10%			−
フェニルアラニン脱アミノ酵素			−
細胞壁アミノ酸	リジン	オルニチン	

【0012】上記の菌学的性質に基づき、バージーズ・マニュアル・オブ・システマチック・バクテリオロジー(Bergey's Manual of Systematic Bacteriology)の記述に従って、前記3C1.7、C1.13、4C1.5の各菌株を次のように同定した

【0013】すなわち、3C1.7株は、グラム陽性、短桿菌(0.6・1μm)、黄色色素産生、胞子の形成なし、運動性なし、通性嫌気性、オキシダーゼ陰性、各種炭水化物の資化能なし、細胞の経時的形態変化において球菌－桿菌の生活環が観察される。また、グルコースからの酸の生成は見られず、細胞壁にはリジンを含むことからアルスロバクター属に属する。また、デンプンを加水分解しないこと、ニコチンおよびヒスチジノールの資化性がないこと、2－ヒドロキシピリジン寒天上で緑色の色素を呈さないことから、アルスロバクター・ウレアファシエンスと同定した。

【0011】C1.13株は、グラム陽性、短桿菌(0.6・2μm)、胞子の生成なし、好気性、オキシダーゼ陰性、各種炭水化物の資化能なし、黄色色素産生、運動性なし、細胞壁アミノ酸分析よりジアミノピメリン酸を

含まずオルニチンを含むこと、デンプンを加水分解しないこと、グルコースからの酸を産生しないこと、１６Ｓｒ ＤＮＡの配列解析からミクロバクテリウム・ルテオラムに対して９５．８％と高い相同性を示した。しかし、同一の性質を示す種が知られていないことから、ミクロバクテリウム属に属する一細菌として、ミクロバクテリウム・スピーシズと同定した。

【００１５】４０１５株は、グラム陰性、短桿菌（０．８×１μｍ）、胞子の生成なし、運動性あり、通性嫌気性、カタラーゼ陽性、グルコースを発酵的に分解し酸およびガスを産生する。オキシダーゼ陰性、クエン酸の利用性あり、インドール産生あり、硫化水素産生なし、アセトイン産生あり、グルコース、Ｄ－マンニトール、イノシトール、Ｄ－ソルビトール、Ｌ－ラムノースなどの各種炭水化物の資化能あり、マロン酸塩の利用性がない、マルトースおよびトレハロースから酸を産生、アセトインを生成することから、エルヴィニア・カロトボラと同定した。

【００１６】なお、これらの菌株に変異を生じさせて一層生産性の高い菌株を得ることもできる。また、これらの菌株の細胞中に存在するエピクロロヒドリンの分解に関与する遺伝子を切り出し、これを適切なベクター例えばプラスミドに挿入し、このベクターを用いて適当な宿主、例えばエッシェリッヒア・コリ（Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ）や酵母のごとき異種宿主もしくはアルスロバクター属、ミクロバクテリウム属、あるいはエルヴィニア属細菌のごとき同種宿主を形質転換することにより、本発明のエピクロロヒドリン分解株を人為的に創成することもできる。

【００１７】（２）微生物の培養方法

前記の微生物を培養して本発明のエピクロロヒドリン分解活性株を製造しようとする場合、基礎栄養培地として、この発明の微生物が増殖し得るものであればいずれを使用してもよい。この培地は、窒素源として例えば硫安、酵母エキス、ペプトン、肉エキス等の１種類又は複数種類を含有する。また、この培地には必要に応じて炭素源としてグルコース、デンプン、グリセリン等を加えることができる。この培地には無機塩類、例えばリン酸二カリウム、塩化ナトリウム、硫酸マグネシウム等を加えることが好ましい。また、酵素の誘導物質となりうる少量のエピクロロヒドリン等、エポキシド化合物を添加することも好ましい。エピクロロヒドリンの添加量は、基礎培地の組成、培養する菌株の性質により異なるが、およそ０．０１～５％である。

【００１８】培養は固体培地又は液体培地のいずれを用いてもよいが、高活性株を多量に得るためには、液体培地を用い、振盪培養、通気・撹拌培養等により好気的条件下で培養を行うのが好ましい。培養温度は菌が生育し、エピクロロヒドリンが分解される温度範囲内であればいずれの温度でも良いが、好ましくは２５～４５℃である。ｐＨは５～１１、好ましくは６～１０の範囲である。培養時間は酵素活性が発現される時間を選べば良いが好ましくは６～７２時間である。

【００１９】細菌菌体の様態としては、特に制限はないが、細胞を含有する培養液、エピクロロヒドリン分解酵素源を含む処理物、培養上清液、培養上清液又は、培養液から分離した菌体の処理物、これから得た酵素剤、さらに、これらの酵素又は、酵素含有物を常法によって固定化したもの等、酵素反応手段として実施される方法であれば反応に供することができる。

【００２０】（３）エピクロロヒドリンの分解

エピクロロヒドリンの分解の様態については、特に制限はないが、通常は前記の細菌菌体を含む反応液に基質としてのエピクロロヒドリン、及び水が含まれていれば反応が進行する。

【００２１】原料のエピクロロヒドリンは反応を阻害しない程度であれば、反応液中の細菌菌体の濃度等により異なり特に限定されないが、１～５００ｇ／Ｌとするのが便利である。エピクロロヒドリンはバッチ式反応においては反応開始時に一度に添加することもでき、又反応の進行と共に複数回に分割して、もしくは連続的に添加することもできる。

【００２２】反応媒体としては、水、又は、アセトン、アセトニトリル、ジメチルスルホキシド、ジメチルホルムアミド等を含む水性液、例えば、水性緩衝液を用いることができる。緩衝液としては、例えば、トリス－塩酸緩衝液、リン酸カリウム緩衝液等を使用することができる。また、ケトン、エーテル、炭化水素、芳香族オレフィン、ハロゲン化炭化水素、有機酸エステル、アルコール、ニトリル等水と混合しない有機溶媒をも用いることもできる。例えば、メチルブチルケトン、イソプロピルエーテル、石油エーテル、ヘキサン、ヘプタン、シクロヘキサン、四塩化炭素、クロロフォルム、二塩化メチレン、トリクロロエタン、ベンゼン、トルエン、キシレン、酢酸エチル、酢酸ブチル、ブタノール、ヘキサノール、オクタノール等を使用することができる。また、それらの有機溶媒の混合物を使うこともできるし、水を飽和させた有機溶媒、水性緩衝液との二層系あるいは、ミセル、逆ミセル、エマルジョンとして反応させることもできる。

【００２３】反応のｐＨとしては、ｐＨ５～１１、好ましくはｐＨ６～１０とする。反応の温度も反応のｐＨと同様に考えることができるが、通常は２０～６０℃、好ましくは２５～５０℃である。反応時間は、特に限定されないが、反応混合物の基質濃度、酵素力価等、に依存して基質エピクロロヒドリンが充分に分解されるまで反応を維持する。

【００２４】

【実施例】次に実施例によりこの発明をさらに具体的に説明する。

【００２５】実施例１
ペプトン０．５％、ＮａＣｌ０．３％、肉エキス０．３％を含有し、ｐＨ７．０に調製したＮｕｔｒｉｅｎｔ培地５ｍＬを試験管に入れ、１２０℃、１５分間加熱殺菌した後、エピクロロヒドリンを０．５から５００ｍＭとなるように加え、それぞれ、アルスロバクター・ウレアファシエンスＳＣＬ７（ＦＥＲＭ　Ｐ－１７４５０）、ミクロバクテリウム・スピーシズＣＬ１３（ＦＥＲＭ　Ｐ－１７４５２）、エルヴィニア・カロトボラ４ＣＬ５（ＦＥＲＭ　Ｐ－１７４５１）を接種し３０℃で２日間振盪培養した。アルスロバクター・ウレアファシエンスＳＣＬ７は、３０ｍＭまで、ミクロバクテリウム・スピーシズＣＬ１３は、２０ｍＭまで、エルヴィニア・カロトボラ４ＣＬ５は、２４ｍＭまでのエピクロロヒドリンでの生育が認められた。
【００２６】実施例２
K_2HPO_4　０．５０％、KH_2PO_4　０．２４％、$(NH_4)_2SO_4$　０．１％、食塩０．１％、$MgSO_4 \cdot 7H_2O$　０．０２％、酵母エキス０．０１％、ビタミン混液、微量金属塩を含有し、ｐＨ７．０に調製した培地０．４Ｌを１２０℃、１５分間加熱殺菌した後、エピクロロヒドリンを１０ｍＭとなるように（３７０ｍｇ　０．４Ｌ）加え、アルスロバクター・ウレアファシエンスＳＣＬ７（ＦＥＲＭ　Ｐ－１７４５０）を接種し３０℃で振盪培養した。培地中の塩素イオンの濃度は、Ｉｗａｓａｋｉの方法（Ｉｗａｓａｋｉ他、Ｂｕｌｌ．　Ｃｈｅｍ．　Ｓｏｃ．　Ｊａｐａｎ，　２５，　２５６　（１９５２）．）により測定した。培地中に含まれていた１０ｍＭのエピクロロヒドリンは培養経過と共に減少し、約４０時間で完全に消失した。菌の生育は、約１００時間で最高になった。培地中の塩素イオン濃度は約１５０時間後に最高になり、１０ｍＭに達した。
【００２７】実施例３
実施例２と同様の培地を調製し、アルスロバクター・ウレアファシエンスＳＣＬ７（ＦＥＲＭ　Ｐ－１７４５０）を接種し３０℃で振盪培養した。０．４Ｌの培地からの菌体を生理的食塩水で洗浄した後、０．１Ｍリン酸緩衝液（ｐＨ７．０）４０ｍＬに懸濁し、エピクロロヒドリンを１０ｍＭなるよう添加して、３０℃で１６０ｒｐｍで振盪した。１０ｍＭ含まれていたエピクロロヒドリンは時間経過と共に減少し、約１４０分間で完全に消失した。
【００２８】実施例４
実施例２と同様の培地を調製し、ミクロバクテリウム・スピーシズＣＬ１３（ＦＥＲＭ　Ｐ－１７４５２）を接種し３０℃で振盪培養した。培地中に含まれていた１０ｍＭのエピクロロヒドリンは培養経過と共に減少し、約１００時間で完全に消失した。菌の生育は、約１００時間で最高になった。培地中の塩素イオン濃度は約１２０時間後に最高になり、１０ｍＭに達した。
【００２９】実施例５
実施例２と同様の培地を調製し、ミクロバクテリウム・スピーシズＣＬ１３（ＦＥＲＭ　Ｐ－１７４５２）を接種し３０℃で振盪培養した。０．４Ｌの培地からの菌体を生理的食塩水で洗浄した後、０．１Ｍリン酸緩衝液（ｐＨ７．０）４０ｍＬに懸濁し、エピクロロヒドリンを１０ｍＭとなるよう添加して、３０℃で１６０ｒｐｍで振盪した。１０ｍＭ含まれていたエピクロロヒドリンは時間経過と共に減少し、約７０分間で完全に消失した。
【００３０】実施例６
実施例２と同様の培地を調製し、エルヴィニア・カロトボラ４ＣＬ５（ＦＥＲＭ　Ｐ－１７４５１）を接種し３０℃で振盪培養した。培地中に含まれていた１０ｍＭのエピクロロヒドリンは培養経過と共に減少し、約８０時間で完全に消失した。菌の生育は、約１５０時間で最高になった。培地中の塩素イオン濃度は約１２０時間後に最高になり、５ｍＭに達した。
【００３１】実施例７
実施例２と同様の培地を調製し、エルヴィニア・カロトボラ４ＣＬ５（ＦＥＲＭ　Ｐ－１７４５１）を接種し３０℃で振盪培養した。０．４Ｌの培地からの菌体を生理的食塩水で洗浄した後、０．１Ｍリン酸緩衝液（ｐＨ７．０）４０ｍＬに懸濁し、エピクロロヒドリンを１０ｍＭとなるよう添加して、３０℃で１６０ｒｐｍで振盪した。１０ｍＭ含まれていたエピクロロヒドリンは時間経過と共に減少し、約１５分間で完全に消失した。
【００３２】
【発明の効果】本発明によってもたらされる新規なエピクロロヒドリンの分解のための強力な新規微生物及びその微生物を利用するエピクロロヒドリンの分解、特に排水、廃液中に含有されるエピクロロヒドリンの効率良い分解方処理が可能となる。

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Abstract:
PROBLEM TO BE SOLVED: To provide a new kind of potent microorganisms intended for

biodegrading epichlorohydrin, and a method for biodegrading epichlorohydrin using the above microorganisms, in particular for degrading epichlorohydrin contained in effluents and waste liquors. SOLUTION: This method for biodegrading epichlorohydrin comprises using a strain selected from each new kind of microorganisms Arthrobacter ureafaciens 3CL7 (FERM P-17450) strain, Microbacterium sp. CL13 (FERM P-17452) and Erwinia carotovora 4CL5 (FERM P-17451) strain.

JPO Machine translation abstract:

(57) **Abstract**

SUBJECT Offer of the method of decomposing the epichlorohydrin contained in decomposition of epichlorohydrin using the powerful new microorganism for decomposition of epichlorohydrin, and its microorganism especially wastewater, and waste fluid.

Means for Solution Microorganism Arthrobacter urea FASHI nth 3CL7(Arthrobacter ureafaciens 3C L7)FERM P-17450 new strain, Micro bacterium species CL13(Microbacterium sp. CL13)FERM P-17452 strain or Elvey Nia KAROTOBORA 4CL5(Erwinia carotovora 4CL5)FERM. A decomposing method of epichlorohydrin using a strain chosen from P-17451 strain.

Claim(s)

Claim 1 Arthrobacter urea FASHI nth 3CL7(Arthrobacter ureafaciens 3C L7)FERM P-17450 strain, Micro bacterium species CL13(Microbacterium sp. CL13)FERM P-17452 strain or Elvey Nia KAROTOBORA 4CL5(Erwinia carotovora 4CL5)FERM. A decomposing method of epichlorohydrin using a strain chosen from P-17451 strain.

Claim 2 Arthrobacter urea FASHI nth 3CL7(Arthrobacter ureafaciens 3C L7)FERM P-17450 strain.

Claim 3 Micro bacterium species CL13(Microbacteriumsp. CL13)FERM P-17452 strain.

Claim 4 Elvey Nia KAROTOBORA 4CL5(Erwinia carotovora 4CL5)FERMP-17451 strain.

Detailed Description of the Invention

0001

Field of the Invention This invention relates to how to process the wastewater containing the epichlorohydrin discharged from the process of using manufacture of epichlorohydrin, or epichlorohydrin. Epichlorohydrin is manufactured and consumed in large quantities as synthetic powder of a chemistry article.

0002

Description of the Prior Art There is special difficulty in processing on the industrial scale of an organic compound with combination of halogen carbon like epichlorohydrin. That is, since the carbon-halogen covalent bond is stable, it is taking great cost to cut this. The organic substance these-halogenated conventionally is disassembled by chemical, a physical method, and the biological method. As a physical method used here, they are the adsorption by activated carbon, and an extraction method. However, this method has the problem that a lot of activated carbon and extracts which were polluted with the halogenated organic compound arise, and requires great expense for these processings. Next, it is the method of decomposing an elevated temperature in oxidative atmosphere and disassembling a halogenated compound on high voltage conditions as chemical processing, in many cases. For example, biological treatment is performed for wastewater after thermal alkali treatment using gram negative bacteria, such as gram positive bacteria, such as the Cellulomonas bacteria, and the Alcaligenes bacteria, as shown in JP,H6-320194,A and a U.S. Pat. No. 5478472 item gazette. The case is performing chemical oxidation treatment. However, by this method, the upper energy cost which needs special equipment is large, and cannot call it an economical approach. After electrolyzing to JP,S50-032767,A, how to process by an ion-exchange membrane is also shown. ****** needs many energies similarly. In addition, there is the method of processing with metal or metal hydride with an organic halogenated compound and high reactivity, and cost cannot say this that cracking severity is also enough high again, either.

0003 As mentioned above, under the present circumstances, since there is no method of decomposing economical outstanding epichlorohydrin, it is obliged to incineration processing of waste fluid using great energy. However, generating of dioxin has been a globally big problem like

recent years, burning is also difficult, and environment was asked for the economical approach with little load.

0004 Mulliken and others (Eur. J. Biochem. 202 and 1217 (1991)), Although Agrobacterium radio Baktar (Agrobacterium radiobacter AD1) has reported growing epichlorohydrin as a single carbon source, it differs from the microorganism of this invention. The culture concentration of epichlorohydrin is to 5mM. Kasai and others (Agric. Biol. Chem. 54. 3185 (1990)) has single Pseudomonas sp. (Pseudomonas sp. OS-K-29) in epichlorohydrin. Although it has said that it grows as a carbon source, It differs from the microorganism of this invention. The culture concentration of epichlorohydrin is up to about 25 mM(s) (0.2%).

0005

Problem to be solved by the invention The purpose of this invention is to provide the method of decomposing the epichlorohydrin contained in decomposition of epichlorohydrin using the powerful new microorganism for decomposition of epichlorohydrin, and its microorganism especially wastewater, and waste fluid.

0006

Means for solving problem In order for this invention person to develop the new biochemical disposal method of epichlorohydrin, when a strain was broadly screened in a nature, he found out that some bacteria decomposed epichlorohydrin. That is, the new strain which decomposes high-concentration epichlorohydrin is acquired out of soil of Yamaguchi Prefecture in Japan, and it is solved by providing a biochemical disposal method of epichlorohydrin by contacting these strains to an aqueous medium containing epichlorohydrin.

0007

Mode for carrying out the invention Hereafter, this invention is explained in detail.

(1) The 3CL7 share, 13 shares of CLs, and five shares of 4CLs which were newly separated in microorganism this invention are screened, are isolated from soil in Yamaguchi, and have high epichlorohydrin decomposition activity. These new strains are shown below.

0008 Seven shares of Arthrobacter urea FASHI nth 3CLs are deposited with National Institute of Bioscience and Human-Technology as FERM P-17450.

0009 Micro bacterium species CL13 is deposited with National Institute of Bioscience and Human-Technology as FERM P-17452.

0010 Elvey Nia KAROTOBORA 4CL5 is deposited with National Institute of Bioscience and Human-Technology as FERM P-17451. Below, the mycology character of these new strains is shown.

0011

5 shares of 13 shares of 3CL seven-share CLs 4CL (a) morphometrical characteristic . form of one cell Bacillus Bacillus the size (micrometer) of Bacillus 2 cell -- 0.6x1 0.6x2 0.8x1 Existence + of 2 pleiomorphia + - Existence - of 3 motility - + Existence - of 4 spore - -(b) culture character -- 1 bouillon agar plate culture (for 30 ** and three days)

b) colony form (a diameter.) mm) 2 2 Three Form of a RO colony Circular . Circular Circular Form of the surface of the Ha colony Smooth . Smooth Smooth Upheaval state of a NI colony Low-convex-like . The shape of low convex The shape of low convex Periphery of a HO colony Entire fringe smooth . entire fringe -- smooth entire fringe -- smooth Color tone of a HE colony Yellow Yellow cream Transparency of a TO colony Opaque Opaque Opaque Gloss of a CHI colony **** **** dull gloss Generation of the Li soluble pigment Nothing Nothing Nothing 2 bouillon agar slant culture (for 30 ** and three days)

b) a quality of growth Fitness Fitness Fitness Gloss of a RO colony **** **** **** -- 3 bouillon liquid culture (for 30 ** and seven days)

b) surface growth **** **** **** RO turbidity It becomes muddy. It becomes muddy. It becomes muddy. The Ha precipitate Powder Powder Powder The NI generation of gas Nothing Nothing Nothing 4 bouillon gelatin (for 30 ** and seven days)

Gelatin liquefaction +5 litmus milk - - (30 **) A blue stain during seven days Erythrochromia Blue stain (c) physiological property . 1 Gram's stain + + - Two Nitrate's reduction . - - + Three Denitrification reaction - - -4. MR test - - - Five VP test . - - + Six Indore generation - -. + Seven Generation **of hydrogen sulfide** - - - 8. Hydrolysis **of starch** - - - 9 citrate use . b) Koser+ + + RO Christensen. + + + Ten Generation of a pigment I King. A culture-medium - - - RO King B culture-medium . - - - 11 Urease - - -. 12 Oxidase - - - 13 Catalase . + + +14 The range of growth I pH 5-9. 6-9 6-8 RO temperature 30 **+ +. + 37 **+ - - 41 **+ -. - 15 An attitude against

oxygen Common gender anaerobiosis . Aerotropism A common gender anaerobic 16 O-F test (glucose). oxidative -- oxidative Generation of acid from fermentation 17 sugars, and gas Acid Gas Acid Gas Acid Gas 1 L-arabinose - - - - + - 2 D-xylose - - - - + - 3 D-glucose - - - - + + 4 D-mannose -. - - - + - 5 D-fructose . - - - -+ - 6 D-galactose . - - - - + - Seven Malt sugar . - - - - + - Eight Shook sirloin . - - + - + - Nine Lactose . - - - - + - Ten Trehalose . - - - - + - 11 D-sorbitol . - - - - + - 12 D-mannitol . - - - - + - 13 Glycerin . - - - - + - 14 Starch . - - - - + - 15 Raffinose . - - - - + - 16 Inulin . - - - - + - 17 D-ribose . - - - - + - 18 Sorbose . - - + - + - 19 Carboxymethyl cellulose . - - - - - - 20 Glycogen . - - - - - Other -(d) and many character . Vitamin demand nature Nothing It makes and nothing Disassembly **of arginine** . + + - Decomposition **of histidinol** - - - Disassembly **of nicotine** - - - Salt tolerance 5%+ - - + 7%+ - - 10% - - - Phenylalanine deaminase - - - Cell wall amino acid Lysine. ornithine**0012**Based on the above-mentioned mycology character, according to description of a bar JIZU manual OBU SHISUTEMA tic bacteriology (Bergey's Mannual of Systematic Bacteriology), Each strain of said 3C L7, CL13, and 4CL5 was identified as follows.

0013 Namely, as for 3CL7 share, the life cycle of a micrococcus-Bacillus is observed in a Gram positive, a short Bacillus (0.6x1 micrometer), yellow-coloring-matter production, the formation nothing of a spore, motile nothing one, a common gender anaerobiosis, oxidase negativity, the utilization incompetence of various carbohydrates, and the temporal shape change of a cell. Generation of acid from glucose is not seen, but since lysine is included in a cell wall, it belongs to Arthrobacter. Arthrobacter urea FASHI since there is **** on that there is no assimilation of not hydrolyzing starch, nicotine, and histidinol, and 2-hydroxypyridine agar about a green pigment as for nothing -- it identified nth.

001413 shares of CLs have a Gram positive, a short Bacillus (0.6x2 micrometers), the generation nothing of a spore, aerotropism, oxidase negativity, the utilization incompetence of various carbohydrates, yellow-coloring-matter production, and no motility. 95.8% and high homology were shown to micro bacterium RUTEORAMU from the sequence analysis of that diaminopimelic acid is not included but ornithine is included from cell wall amino acid analysis, not hydrolyzing starch, not producing acid from glucose, and 16S rDNA. However, since the kind in which the same character is shown was not known, the micro bacterium species was identified as one bacteria belonging to Microbacterium.

0015 Five shares of 4CLs decompose Gram negative, a short Bacillus (0.8x1 micrometer), the generation nothing of a spore, motile ****, a common gender anaerobiosis, a catalase positivity, and glucose in fermentation, and produce acid and gas. Oxidase negativity and citrate are available and Those with the Indore production, hydrogen sulfide production nothing, Those with acetoin production, glucose, D-mannitol, inositol, Since acid was produced from malt sugar and trehalose without those of various carbohydrates, such as D-sorbitol and L-rhamnose, with utilization ability, and the availability of chestnut acid chloride and acetoin was generated, Elvey Nia KAROTOBORA was identified.

0016 These strains are made to produce variation and a strain with still higher productivity can also be obtained. The gene which participates in decomposition of the epichlorohydrin which exists in the cell of these strains is started, Insert this in suitable vector, for example, plasmid, and using this vector A suitable host, For example, the different-species host or Arthrobacter like an ESSHIERIHHIA Coli (Escherichia coli) or yeast, The epichlorohydrin decomposition stock of this invention can also be artificially created by transforming the host of the same kind like Microbacterium or genus-erwinia bacteria.

0017(2) When the microorganism of the culturing method above of a microorganism tends to be cultivated and it is going to manufacture the epichlorohydrin decomposition activity stock of this invention, as long as the microorganism of this invention may propagate, any may be used as a basic nutrient medium. This culture medium contains one kind, such as ammonium sulfate, a yeast extract, peptone, and a meat extract, or two or more kinds as a nitrogen source. Glucose, starch, glycerin, etc. can be added to this culture medium as a carbon source if needed. It is preferred to add mineral, for example, phosphoric acid dipotassium, sodium chloride, magnesium sulfate, etc. to this culture medium. It is also preferred to add an epoxide compound, such as epichlorohydrin etc. of a small quantity which can serve as an inductor of an enzyme. Although the addition of epichlorohydrin changes with the presentation of a basal medium, and character of the strain to cultivate, it is about 0.01 to 5%.

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hyperactive stock so much, it is preferred to cultivate under aerobic conditions by shaking culture, aeration, spinner culture, etc. using a liquid medium. As long as it is in the temperature requirement where a bacillus grows culture temperature and epichlorohydrin is decomposed, which temperature may be sufficient, but it is 25-45 ** preferably. pH -- 5-11 -- it is the range of 6-10 preferably. Although the culture time should just choose the time when enzyme activity is revealed, it is 6 to 72 hours preferably.

0019 The treating material, culture supernatant fluid, culture supernatant fluid which include the culture medium containing a cell, and the source of an epichlorohydrin dialytic ferment as aspect of a bacteria biomass although there is no restriction in particular. Or further, if the treating material of the biomass separated from culture medium, the enzyme agent obtained from this, these enzymes or the thing which fixed the enzyme inclusion with the conventional method, etc. is the methods enforced as an enzyme reaction means, a reaction can be presented.

0020(3) Although there is no restriction in particular about the aspect of decomposition of decomposition epichlorohydrin of epichlorohydrin, if epichlorohydrin as a substrate and water are contained in the reaction mixture which usually contains the aforementioned bacteria biomass, a reaction will advance.

0021 If epichlorohydrin of a raw material is a grade which does not check a reaction, it will especially change with concentration of the bacteria biomass in reaction mixture, etc., and will not be limited, but it is convenient to consider it as 1-500 g/L. In a batch type reaction, it can also add at once at the time of a reaction start, and epichlorohydrin can be divided into multiple times with advance of a reaction, or can also be added continuously.

0022 As a reaction medium, the aquosity liquid, for example, an aqueous buffer, containing water or acetone, acetonitrile, dimethyl sulfoxide, dimethylformamide, etc. can be used. As buffer solution, tris-chloride buffer solution, potassium phosphate buffer solution, etc. can be used, for example. The organic solvent which is not mixed with water, such as ketone, ether, hydrocarbon, an aromatic olefin, halogenated hydrocarbon, organic acid ester, alcohol, and nitril, can also be used. For example, methyl butyl ketone, isopropyl ether, petroleum ether, Hexane, heptane, cyclohexane, a carbon tetrachloride, chloroform, a methylene dichloride, trichloroethane, benzene, toluene, xylene, ethyl acetate, butyl acetate, butanol, a hexanol, octanol, etc. can be used. The mixture of those organic solvents can also be used and it can also be made to react as a bilayer system with the organic solvent and aqueous buffer which saturated water or micell, reversed micelle, and an emulsion.

0023as pH of a reaction -- pH 5-11 -- it is preferably referred to as pH 6-10. Although the temperature of a reaction can be considered to be pH of a reaction the same way, 20-60 ** is usually 25-50 ** preferably. Although reaction time in particular is not limited, it maintains a reaction until it depends without the substrate concentration of a reaction mixture, enzymatic-activity value, etc. and substrate epichlorohydrin is fully decomposed.

0024

Working example Next, an embodiment explains this invention still more concretely.

0025 NaCl0.3% and 0.3% of a meat extract are contained embodiment 1 peptone 0.5%, Nutrient culture-medium 5mL prepared to pH 7.0 is put into a test tube, After heat-sterilizing for 15 minutes, 120 ** of epichlorohydrin is added so that it may be set to 500mM from 0.5, Respectively, Arthrobacter urea FASHI nth 3C L7 (FERM P-17450) and micro bacterium species CL13 (FERM P-17452) and Elvey Nia KAROTOBORA 4CL5 (FERMP-17451) were inoculated, and shaking culture was carried out for two days at 30 **. As for Elvey Nia KAROTOBORA 4CL5, as for Arthrobacter urea FASHI nth 3C L7, growth by epichlorohydrin to 24mM was accepted to 26mM micro bacterium species CL13 to 30mM.

0026 Embodiment 2K $_2HPO_4$0.56%, $KH_2PO_4$0.24%, $(NH_4)_2SO_4$0.1%, 0.1% of salt, $MgSO_4$ and 7H $_2$O0.02%, 0.01% of a yeast extract, The culture medium 0.4L which contained vitamin mixture and a trace element salt and was prepared to pH 7.0 120 **, After heat-sterilizing for 15 minutes, epichlorohydrin was added so that it might be set to 10mM (370mg/0.4L), Arthrobacter urea FASHI nth 3C L7 (FERM P-17450) was inoculated, and shaking culture was carried out at 30 **. The concentration of the chloride ion in a culture medium was measured by the method (25, Bull. Chem. Soc. Japan, 256. (1952) besides Iwasaki) of Iwasaki. Epichlorohydrin of 10mM contained in the culture medium decreased with culture progress, and disappeared thoroughly in about 40

hours. Growth of the bacillus became the highest in about 100 hours. The chloride-ion concentration in a culture medium became the highest about 150 hours afterward, and reached 10mM.

0027 The same culture medium as embodiment 3 Embodiment 2 was prepared, Arthrobacter urea FASHI nth 3C L7 (FERM P-17450) was inoculated, and shaking culture was carried out at 30 **. 0. After physiological sodium chloride solution washed the biomass from the culture medium of 4L, it suspended to 0.1M phosphate buffer solution (pH 7.0) 40mL, and epichlorohydrin was added so that it might become 10 mM, and it shook at 160 rpm at 30 **. The epichlorohydrin contained 10 mM decreased with time progress, and disappeared thoroughly in about 140 minutes.

0028 The same culture medium as embodiment 4 Embodiment 2 was prepared, micro bacterium species CL13 (FERM P-17452) was inoculated, and shaking culture was carried out at 30 **. Epichlorohydrin of 10mM contained in the culture medium decreased with culture progress, and disappeared thoroughly in about 100 hours. Growth of the bacillus became the highest in about 100 hours. The chloride-ion concentration in a culture medium became the highest about 120 hours afterward, and reached 10mM.

0029 The same culture medium as embodiment 5 Embodiment 2 was prepared, micro bacterium species CL13 (FERM P-17452) was inoculated, and shaking culture was carried out at 30 **. 0. After physiological sodium chloride solution washed the biomass from the culture medium of 4L, it suspended to 0.1M phosphate buffer solution (pH 7.0) 40mL, and epichlorohydrin was added so that it might be set to 10mM, and it shook at 160 rpm at 30 **. The epichlorohydrin contained 10 mM decreased with time progress, and disappeared thoroughly in about 70 minutes.

0030 The same culture medium as embodiment 6 Embodiment 2 was prepared, Elvey Nia KAROTOBORA 4CL5 (FERM P-17451) was inoculated, and shaking culture was carried out at 30 **. Epichlorohydrin of 10mM contained in the culture medium decreased with culture progress, and disappeared thoroughly in about 80 hours. Growth of the bacillus became the highest in about 150 hours. The chloride-ion concentration in a culture medium became the highest about 120 hours afterward, and reached 5mM.

0031 The same culture medium as embodiment 7 Embodiment 2 was prepared, Elvey Nia KAROTOBORA 4CL5 (FERM P-17451) was inoculated, and shaking culture was carried out at 30 **. 0. After physiological sodium chloride solution washed the biomass from the culture medium of 4L, it suspended to 0.1M phosphate buffer solution (pH 7.0) 40mL, and epichlorohydrin was added so that it might be set to 10mM, and it shook at 160 rpm at 30 **. The epichlorohydrin contained 10 mM decreased with time progress, and disappeared thoroughly in about 15 minutes.

0032

Effect of the Invention The efficient method processing of decomposition of the epichlorohydrin contained in decomposition of epichlorohydrin using the powerful new microorganism for decomposition of the new epichlorohydrin brought about by this invention and its microorganism especially wastewater, and waste fluid is attained.

Field of the Invention This invention relates to how to process the wastewater containing the epichlorohydrin discharged from the process of using manufacture of epichlorohydrin, or epichlorohydrin. Epichlorohydrin is manufactured and consumed in large quantities as synthetic powder of a chemistry article.

Description of the Prior Art There is special difficulty in processing on the industrial scale of an organic compound with combination of halogen carbon like epichlorohydrin. That is, since the carbon-halogen covalent bond is stable, it is taking great cost to cut this. The organic substance these-halogenated conventionally is disassembled by chemical, a physical method, and the biological method. As a physical method used here, they are the adsorption by activated carbon, and an extraction method. However, this method has the problem that a lot of activated carbon and extracts which were polluted with the halogenated organic compound arise, and requires great expense for these processings. Next, it is the method of decomposing an elevated temperature in

oxidative atmosphere and disassembling a halogenated compound on high voltage conditions as chemical processing, in many cases. For example, biological treatment is performed for wastewater after thermal alkali treatment using gram negative bacteria, such as gram positive bacteria, such as the Cellulomonas bacteria, and the Alcaligenes bacteria, as shown in JP,H6-320194,A and a U.S. Pat. No. 5478472 item gazette. The case is performing chemical oxidation treatment. However, by this method, the upper energy cost which needs special equipment is large, and cannot call it an economical approach. After electrolyzing to JP,S50-032767,A, how to process by an ion-exchange membrane is also shown. ****** needs many energies similarly. In addition, there is the method of processing with metal or metal hydride with an organic halogenated compound and high reactivity, and cost cannot say this that cracking severity is also enough high again, either.

0003 As mentioned above, under the present circumstances, since there is no method of decomposing economical outstanding epichlorohydrin, it is obliged to incineration processing of waste fluid using great energy. However, generating of dioxin has been a globally big problem like recent years, burning is also difficult, and environment was asked for the economical approach with little load.

0004 Mulliken and others (Eur. J. Biochem. 202 and 1217 (1991)), Although Agrobacterium radio Baktar (Agrobacterium radiobacter AD1) has reported growing epichlorohydrin as a single carbon source, it differs from the microorganism of this invention. The culture concentration of epichlorohydrin is to 5mM. Kasai and others (Agric. Biol. Chem. 54. 3185 (1990)) has single Pseudomonas sp. (Pseudomonas sp. OS-K-29) in epichlorohydrin. Although it has said that it grows as a carbon source, It differs from the microorganism of this invention. The culture concentration of epichlorohydrin is up to about 25 mM(s) (0.2%).

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suspended to 0.1M phosphate buffer solution (pH 7.0) 40mL, and epichlorohydrin was added so that it might become 10 mM, and it shook at 160 rpm at 30 **. Epichlorohydrin contained 10 mM decreased with time progress, and disappeared thoroughly in about 140 minutes.
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Problem to be solved by the invention The purpose of this invention is to provide the method of decomposing the epichlorohydrin contained in decomposition of epichlorohydrin using the powerful new microorganism for decomposition of epichlorohydrin, and its microorganism especially wastewater, and waste fluid.

Means for solving problem In order for this invention person to develop the new biochemical disposal method of epichlorohydrin, when a strain was broadly screened in a nature, he found out that some bacteria decomposed epichlorohydrin. That is, the new strain which decomposes high-concentration epichlorohydrin is acquired out of soil of Yamaguchi Prefecture in Japan, and it is solved by providing a biochemical disposal method of epichlorohydrin by contacting these strains to an aqueous medium containing epichlorohydrin.
0007
Mode for carrying out the invention Hereafter, this invention is explained in detail.
(1) The 3CL7 share, 13 shares of CLs, and five shares of 4CLs which were newly separated in microorganism this invention are screened, are isolated from soil in Yamaguchi, and have high epichlorohydrin decomposition activity. These new strains are shown below.
0008 Seven shares of Arthrobacter urea FASHI nth 3CLs are deposited with National Institute of Bioscience and Human-Technology as FERM P-17450.
0009 Micro bacterium species CL13 is deposited with National Institute of Bioscience and Human-Technology as FERM P-17452.
0010 Elvey Nia KAROTOBORA 4CL5 is deposited with National Institute of Bioscience and Human-Technology as FERM P-17451. Below, mycology character of these new strains is shown.
0011
5 shares of 13 shares of 3CL seven-share CLs 4CL (a) morphometrical characteristic . form of one

cell Bacillus Bacillus the size (micrometer) of Bacillus 2 cell -- 0.6x1 0.6x2 0.8x1 Existence + of 2 pleiomorphia + - Existence - of 3 motility - + Existence - of 4 spore - -(b) culture character -- 1 bouillon agar plate culture (for 30 ** and three days)
b) colony form (a diameter.) mm) 2 2 Three Form of a RO colony Circular . Circular Circular Form of the surface of the Ha colony Smooth . Smooth Smooth Upheaval state of a NI colony Low-convex-like . The shape of low convex The shape of low convex Periphery of a HO colony Entire fringe smooth . entire fringe -- smooth entire fringe -- smooth Color tone of a HE colony Yellow Yellow cream Transparency of a TO colony Opaque Opaque Opaque Gloss of a CHI colony **** **** dull gloss Generation of the Li soluble pigment Nothing Nothing Nothing 2 bouillon agar slant culture (for 30 ** and three days)
b) Quality of growth Fitness Fitness Fitness Gloss of a RO colony **** **** It is and is 3 bouillon liquid culture (for 30 ** and seven days).
b) Surface growth **** **** **** RO turbidity It becomes muddy. It becomes muddy. It becomes muddy. Ha precipitate Powder Powder Powder NI generation of gas Nothing Nothing Nothing 4 bouillon gelatin (for 30 ** and seven days)
Gelatin liquefaction +5 litmus milk - - (30 **) Blue stain during seven days Erythrochromia Blue stain (c) physiological property . 1 Gram's stain + + - Two Nitrate's reduction . - - + Three Denitrification reaction - - -4. MR test - - - Five VP test . - - + Six Indore generation - -. + Seven Generation **of hydrogen sulfide** - - - 8. Hydrolysis **of starch** - - - 9 citrate use . b) Koser+ + + RO Christensen. + + + Ten Generation of a pigment I King. A culture-medium - - - RO King B culture-medium . - - - 11 Urease - - -. 12 Oxidase - - - 13 Catalase . + + +14 The range of growth I pH 5-9. 6-9 6-8 RO temperature 30 **+ +. + 37 **+ - - 41 **+ -. - 15 Attitude against oxygen Common gender anaerobiosis . Aerotropism A common gender anaerobic 16 O-F test (glucose). oxidative -- oxidative Generation of acid from fermentation 17 sugars, and gas Acid Gas Acid Gas Acid Gas 1 L-arabinose - - - - + - 2 D-xylose - - - - + - 3 D-glucose - - - - + + 4 D-mannose -. - - - + - 5 D-fructose . - - - -+ - 6 D-galactose . - - - - + - Seven Malt sugar . - - - - + - Eight Shook sirloin . - - + - + - Nine Lactose . - - - - + - Ten Trehalose . - - - - + - 11 D-sorbitol . - - - - + - 12 D-mannitol . - - - - + - 13 Glycerin . - - - - + - 14 Starch . - - - - + - 15 Raffinose . - - - - + - 16 Inulin . - - - - + - 17 D-ribose . - - - - + - 18 Sorbose . - - + - + - 19 Carboxymethyl cellulose . - - - - - - 20 Glycogen . - - - - - Other -(d) and many character . Vitamin demand nature Nothing It makes and nothing Disassembly **of arginine** . + + - Decomposition **of histidinol** - - - Disassembly **of nicotine** - - - Salt tolerance 5%+ - - + 7%+ - - 10% - - - Phenylalanine deaminase - - - Cell wall amino acid Lysine. ornithine**0012**Based on the above-mentioned mycology character, according to description of a bar JIZU manual OBU SHISUTEMA tic bacteriology (Bergey's Mannual of Systematic Bacteriology), Each strain of said 3C L7, CL13, and 4CL5 was identified as follows.
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0020(3) Although there is no restriction in particular about the aspect of decomposition of decomposition epichlorohydrin of epichlorohydrin, if epichlorohydrin as a substrate and water are contained in the reaction mixture which usually contains the aforementioned bacteria biomass, a reaction will advance.

0021 If epichlorohydrin of a raw material is a grade which does not check a reaction, it will especially change with concentration of the bacteria biomass in reaction mixture, etc., and will not be limited, but it is convenient to consider it as 1-500 g/L. In a batch type reaction, it can also add at once at the time of a reaction start, and epichlorohydrin can be divided into multiple times with advance of a reaction, or can also be added continuously.

0022 As a reaction medium, the aquosity liquid, for example, an aqueous buffer, containing water or acetone, acetonitrile, dimethyl sulfoxide, dimethylformamide, etc. can be used. As buffer solution, tris-chloride buffer solution, potassium phosphate buffer solution, etc. can be used, for example. The organic solvent which is not mixed with water, such as ketone, ether, hydrocarbon, an aromatic olefin, halogenated hydrocarbon, organic acid ester, alcohol, and nitril, can also be used. For example, methyl butyl ketone, isopropyl ether, petroleum ether, Hexane, heptane, cyclohexane, a carbon tetrachloride, chloroform, a methylene dichloride, trichloroethane, benzene, toluene, xylene, ethyl acetate, butyl acetate, butanol, a hexanol, octanol, etc. can be used. The mixture of those organic solvents can also be used and it can also be made to react as a bilayer system with the organic solvent and aqueous buffer which saturated water or micell, reversed micelle, and an emulsion.

0023as pH of a reaction -- pH 5-11 -- it is preferably referred to as pH 6-10. Although the temperature of a reaction can be considered to be pH of a reaction the same way, 20-60 ** is

usually 25-50 ** preferably. Although reaction time in particular is not limited, it maintains a reaction until it depends without the substrate concentration of a reaction mixture, enzymatic-activity value, etc. and substrate epichlorohydrin is fully decomposed.